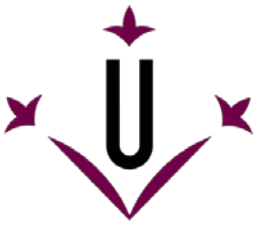

Lack of protective effect of chloroquine derivatives on COVID-19 disease in a Spanish sample of chronically treated patients.

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Running heads: No prophylactic effect of hydroxychloroquine against COVID-19 disease

Abstract

Background: The search for a SARS-CoV-2 treatment has emerged as a worldwide priority. We evaluated the role of chloroquine and its derivatives in COVID-19 in Spanish individuals.

Methods: We performed a survey addressed to patients regularly taking chloroquine and its derivatives for the control of their autoimmune diseases. The survey was distributed with special attention to Spanish patient associations centred on autoimmune diseases and rheumatology and to the general population. A sample of untreated subjects was matched to the treated group according to sex, age range and incidence region. COVID-19 disease prevalence was compared between treated and untreated-matched control sample.

Results: A total of 319 surveys of patients regularly taking chloroquine and its derivatives were recovered for further analysis. The prevalence of declared COVID-19 status in the treated group was 5.3% and the mean prevalence among the untreated-matched groups was 3.4%. A community exposition to COVID-19 was associated with a greater prevalence of COVID-19 in both, treated (17.0% vs. 3.2%; p-value<0.001) and untreated groups (13.4% vs. 1.1%; p-value=0.027).

Conclusion: We did not find differences of reported COVID-19 cases between treated and untreated groups, indicating a lack of protection by regular administration of chloroquine and its derivative drugs on COVID-19 infection. Of relevance, data indicates that patients that regularly take chloroquine derivatives are exposed to SARS-CoV-2 infection and must take the same protection measures as the general population.

Keywords: chloroquine, COVID-19 prevalence, hydroxychloroquine, prophylaxis, SARS-CoV-2

Introduction

The search for an effective treatment against SARS-CoV-2 infection has emerged as a worldwide priority. A promising strategy to fight the virus causing the newest pandemic is the identification of already available drugs active against other diseases that can be effective also against this new SARS-CoV-2 infection [1].

We present here an evaluation of the potential protective effect of chloroquine and its derived drugs on the prevalence of COVID-19 in patients undergoing an active treatment with these drugs.

Chloroquine and its derived drugs such as hydroxychloroquine have shown *in vitro* and *in vivo* effectiveness against viral infections such as SARS [2], influenza A H5N1, and Zika. Chloroquine is believed to interfere with both the entry and exit of viruses from cells hosts, as well as in the manifestation of acute respiratory syndrome. The virus enters the cells through binding to the angiotensin-converting enzyme 2 (ACE2). Chloroquine can reduce ACE2 glycosylation, thereby preventing viruses from effectively binding to cells [3]. On the other hand, chloroquine accumulates in lysosomes, increasing the endosome pH levels which interferes with the viral particle release process [4]. In addition, chloroquine could block the production of proinflammatory cytokines, thus preventing the pathway that subsequently leads to acute respiratory syndrome [5]. Two recent clinical trials have presented inconclusive evidence on the effectiveness of chloroquine treatment in COVID-19 disease in Chinese [6] and French [7] populations. Both studies have supported the use of chloroquine or chloroquine derivatives against COVID-19, however, the design and conclusions of both studies have been questioned [8].

Chloroquine and its derivatives such as chloroquine phosphate or hydroxychloroquine are commonly used in the treatment of autoimmune diseases. Not without serious side effects, the use of these drugs

under medical prescription is widely spread. It has been proposed hydroxychloroquine as a prophylaxis treatment against SARS-CoV-2 infection for exposed caregivers [9]. Our hypothesis is that if chloroquine treatment is effective against SARS-CoV-2 infection, those patients following an active chloroquine or derivative drug treatment would be protected against the infection or against COVID-19 adverse effects. Thus, our study aims to test this hypothesis by evaluating the incidence of COVID-19 disease in the population according to chloroquine treatment subgroups through a survey.

Material and methods

Survey design and data collection

A survey was designed to be conducted electronically via smartphone or personal computer, and therefore it was also designed to ensure accessibility and simplicity to facilitate its completion. Information about the project and a link to the URL of the survey were disseminated in the press and via social media and email, with special attention to Spanish patient associations centered on autoimmune diseases and rheumatology. The survey included demographic questions about gender, age range, and province of residence, as well as questions pertaining to health-status outcomes such as treatment, COVID-19 diagnosis and symptoms due to COVID-19 infection. In addition, questions about infection diagnosis and symptoms in close relatives and friends were also included. Because the first cases of COVID-19 were reported in Spain in March 2020, our survey collects cases that occurred between March and May 2020.

Individuals of any age above legal age (18 years old) with residence in Spain were eligible for inclusion in the study. Individuals undergoing a stable chloroquine or derived drug treatment before

the COVID-19 pandemic were classified as treated (treated group) while individuals without treatment were classified as untreated (untreated group).

The Clinical Research Ethics Committee of the Hospital Universitari Arnau de Vilanova in Lleida approved the study (Ref: CEIC-2257).

Statistical analysis

Statistical analyses were performed with R software (v3.6.0) and IBM SPSS v21 (IBM corporation, NY, USA). Prior to the analysis, reported provinces of residence in Spain were grouped according to COVID-19 incidence as stated in June 1 by the Spanish Ministry of Health [10] as follows: Incidence region 1 (incidence<200/100,000 inhabitants) that includes Andalucía, Canarias, Ceuta, Illes Balears, Melilla and Región de Murcia; Incidence region 2 (incidence 200-500/100,000 inhabitants) that includes Aragón, Cantabria, Comunidad Valenciana, Extremadura, Galicia and Principado de Asturias; Incidence region 3 (incidence 500-1,000/100,000 inhabitants) that includes Castilla y León, Castilla-La Mancha, Catalunya, Euskadi and Navarra; and Incidence region 4 (incidence>1,000/100,000 inhabitants) that includes Comunidad de Madrid and La Rioja. Age categories were assigned according to the following age ranges: 18-50, 51-65 and >65 years old. Data on symptoms related to COVID-19 infection were collected and used to assign individuals as suspected COVID-19 cases when reporting loss of taste or smell and/or three or more COVID-19 associated symptoms [11].

A sample of untreated subjects was matched to the treated group according to sex, age range and incidence region with e1071 R package. The matching process was repeated using a bootstrap strategy and re-sampling of the untreated-matched dataset was repeated 1,000 times to obtain the distribution and mean values of the descriptive statistics such as age range, gender, incidence region

and declared COVID-19 prevalence. Comparisons between groups were performed by Fisher exact test. P-value<0.05 was considered statistically significant.

Results

Overall, 2295 individuals completed the surveys between May and June 2020. From these, data collection was complete, with all key data for the study, for 2161 individuals (94.2%). Of these completed surveys, 11 entries did not meet eligibility criteria and were excluded from the analysis. The final number of completed surveys used for the study was 2150. Among them, 319 (14.8%) were from patients following an active chloroquine or derived drug treatment and have been included in the treatment group, and 1831 (85.2%) have been included in the untreated group and serves as the source to obtain the untreated-matched subgroups. We note that 94% of the treatment group individuals were following a hydroxychloroquine treatment.

The main descriptive characteristics of both treated (n=319) and untreated-matched (n=319) subgroups, arranged according to their declared COVID-19 status, are reported in Table 1. Distribution of declared COVID-19 status did not differ significantly within age group, gender and incidence region (Table 1). In contrast, having a community exposition (defined as those individuals declaring a COVID-19 positive case in a close family member or flatmate) was associated with a greater prevalence of COVID-19 disease when compared with non-exposed individuals in both treated (17.0%, 95%CI 12.9%-21.1% vs. 3.2%, 95%CI 1.3%-5.2%; p-value<0.001) and untreated subgroups (13.4%, 95%CI 9.6%-17.1% vs. 1.1%, 95%CI 0%-2.2%; p-value=0.027) (Table 1 and Fig 1).

Table 1 - Demographic characteristics of persons taking chloroquine or derivatives (treated) and matched control sample (untreated) according to their declared COVID-19 status

	Treated (n=319)				Untreated (n[a] =319)			
	COVID-19 (+)	COVID-19 (-)	Totals	P-value	COVID-19 (+)	COVID-19 (-)	Totals	P-value
Age range, n (%)								
18-50	9 (4.7)	182 (95.3)	191	0.433	6.7 (3.5)	185.4 (96.5)	192.2	0.999
51-65	8 (7.1)	105 (92.9)	113		3.9 (3.5)	107.5 (96.5)	111.3	
> 65	0 (0.0)	15 (100.0)	15		0.3 (2.1)	15.2 (97.9)	15.5	
Total	17 (5.3)	302 (94.7)	319		10.9 (3.4)	308.1 (96.6)	319	
Sex, n (%)								
Male	1 (4.8)	20 (95.2)	21	0.905	1.1 (5.0)	20.2 (95.0)	21.2	0.973
Female	16 (5.4)	282 (94.6)	298		9.8 (3.3)	287.9 (96.7)	297.8	
Total	17 (5.3)	302 (94.7)	319		10.9 (3.4)	308.1 (96.6)	319	
Incidence region[b], n (%)								
R1	1 (2.4)	40 (97.6)	41	0.420	0.0 (0.0)	41.1 (100.0)	41.1	0.275
R2	1 (2.8)	35 (97.2)	36		1.0 (3.4)	28.7 (96.6)	29.7	
R3	11 (5.4)	193 (94.6)	204		6.1 (2.7)	218.8 (97.3)	225.0	
R4	4 (11.4)	31 (88.6)	35		3.2 (15.9)	17.0 (84.1)	20.2	
Unknown	0 (0.0)	3 (100.0)	3		0.5 (17.3)	2.5 (82.7)	3.0	
Total	17 (5.3)	302 (94.7)	319		10.9 (3.4)	308.1 (96.6)	319	
Community exposition, n (%)								
Exposed[c]	8 (17.0)	39 (83.0)	47	<0.001	8.2 (13.3)	53.3 (86.7)	61.5	0.027
Unexposed	7 (3.2)	210 (96.8)	217		2.2 (1.1)	204.4 (98.9)	206.6	
Unknown	2 (3.6)	53 (96.4)	55		0.5 (1.0)	50.4 (99.0)	50.9	
Total	17 (5.3)	302 (94.7)	319		10.9 (3.4)	308.1 (96.6)	319	

COVID-19: disease caused by SARS-CoV-2 infection.

[a] Mean after 1000 replicates of matched untreated control samples.

[b] Regions grouped by incidence of COVID-19. IR1 (incidence <200/100,000 inhabitants) that includes Andalucía, Canarias, Ceuta, Illes Balears, Melilla and Región de Murcia; IR2 (incidence 200-500/100,000 inhabitants) that includes Aragón, Cantabria, Comunidad Valenciana, Extremadura, Galicia and Principado de Asturias; IR3 (incidence 500-1,000/100,000 inhabitants) that includes Castilla y León, Castilla-La Mancha, Catalunya, Euskadi and Navarra; and IR4 (incidence >1,000/100,000 inhabitants) that includes Comunidad de Madrid and La Rioja.

[c] Exposed were those individuals declaring a COVID-19 positive case in a close family member or flatmate.

The prevalence of declared COVID-19 status in the treated group was 5.3% (95%CI 2.9-7.8) and the mean prevalence among the untreated-matched groups was 3.4% (95%CI 1.4-5.4). Furthermore, the prevalence of suspected COVID-19 patients in treated subjects was of 18.8% (95%CI 14.5-23.1) and the mean prevalence among the untreated-matched groups was 15.7% (95%CI 11.7-19.7). These figures are nearly similar to those recently found in the study of the seroprevalence of IgG antibodies

against SARS-CoV-2 in the Spanish population showing an estimated prevalence of 5% (95%CI 4.7%-5.4%), and a prevalence of suspected COVID-19 cases of nearly 20%.[11]

Discussion

Our results show no differences in COVID-19 prevalence among untreated and chronically treated individuals with chloroquine or derivative drugs. Independently of the exposure, both groups showed the same prevalence of COVID-19 disease or suspected COVID-19 disease according to symptoms. We must note that we found a clear association between the COVID-19 disease prevalence and exposure to a close family member or flatmate positive for COVID-19 in both, treated and untreated subjects, that points to a lack of any protective effect on SARS-CoV-2 infection attributable to chronic treatment with chloroquine or derivative drugs.

We should mention some limitations of our study such as a limited power to detect small changes in prevalence between treatment groups. However, the design of this study addressed the need to collect and analyse data within a particularly short period of time due to the rapid onset and progression of the pandemic, as well as the urgency of identifying and evaluating rapidly possible therapies, thus partially compensating the reduced sample size. The prevalence of COVID-19 found in our study is similar to the seroprevalence of IgG antibodies against SARS-CoV-2 in the Spanish population [11], which is higher than the reported COVID-19 prevalence in the general population based on RNA's virus detection [10]. This difference could be attributed to self-reported disease and the diagnosis of COVID-19 by medical practitioners, which in many cases does not involve results of diagnostic tests due to the lack of such tests. Finally, we could not eliminate completely the possibility of some bias due to the intrinsic condition of the individuals within the treatment group that are undergoing chloroquine or derivative drug treatment due to other diseases that alter their health status and may have different comorbidities. However, our results are in line with a recent study conducting a

157 randomized trial that reported no effect of hydroxychloroquine when used as a post exposure
158 prophylaxis for COVID-19 [12].

159 All these data together point towards a lack of a protective effect of chloroquine or derivative drugs
160 as a prophylaxis for COVID-19, including prophylactic treatment before and after exposure. Of
161 relevance, data indicates that people that regularly take chloroquine derivatives are exposed to
162 SARS-CoV-2 infection and must take the same protection measures as the general population. These
163 data should be considered in the prevention and treatment protocols made by health policymakers for
164 the management of the disease in new outbreaks.

165 Acknowledgments

166 This study and the research behind it would not have been possible without the selfless contribution
167 of all the people who participated and that took time to respond the survey. We would like to thank
168 Dr. Serafi (Tapi) Piñol for his valuable comments and suggestions.

169 Author Contributions

170 JF created the online survey, conceived and design the study. ML and JF were responsible for data
171 analysis. All authors were involved in the development of the survey, contributed to the writing of
172 the manuscript and approved the final draft

173 Conflict of Interest

174 The authors declare that the research was conducted in the absence of any commercial or financial
175 relationships that could be construed as a potential conflict of interest.

176 Funding

177 This research did not receive any specific grant from funding agencies in the public, commercial, or

178 not-for-profit sectors.

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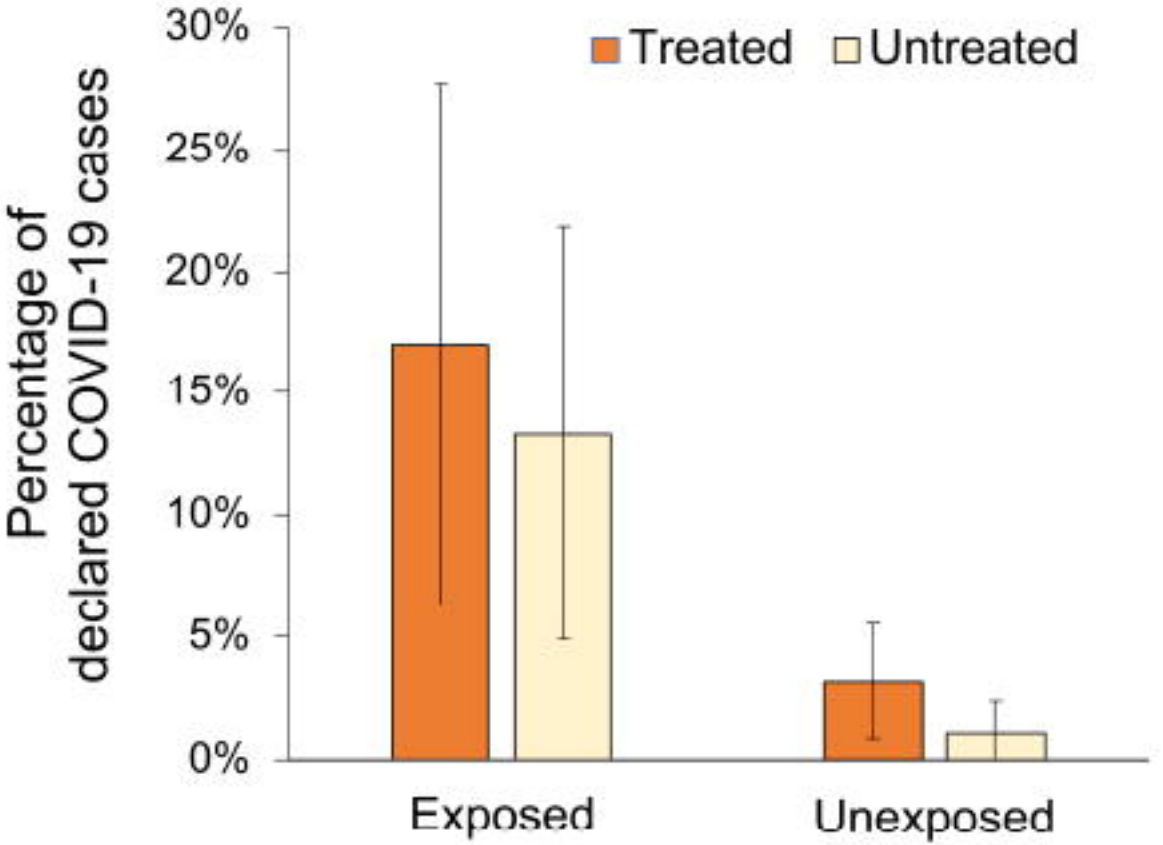

Figure 1.- Percentage of declared COVID-19 cases according to treatment status and community exposition. Treated group refers to patients regularly taking chloroquine and its derivatives. Exposed are those individuals declaring a COVID-19 positive case in a close family member or flatmate. Error bars depict the 95% confidence interval.